library was incubated with biotinylated target and subsequently with streptavidin-coated magnetic beads. The library was screened with CMACS for target-binding peptides and the screened clones were amplified overnight. The fraction of target-binding population in the library was analyzed by flow cytometry after incubating them with fluorescently labeled target.

[0032] FIGS. **10**A and **10**B are diagrams showing a multi-layer buffer switching sorting device having multiple sorting devices operating in parallel.

DESCRIPTION OF CERTAIN EMBODIMENTS

[0033] Introduction

[0034] In accordance with certain embodiments of this invention, a microfluidic device includes one or more magnetic field gradient generators (MFGs) useful for separating magnetic and non-magnetic particles in a continuous-flow system. The microfluidic devices of this invention may employ a laminar flow buffer switching configuration in which separate streams of buffer and sample are directed into separation regions of the microfluidic device. The combination of an MFG with a laminar-flow buffer switching scheme provides an efficient means for suppressing undesired mixing and rejecting non-target components so that the target components are purified with high efficiency. The device performance can be enhanced in some embodiments by providing integrated multiple MFG separation regions. These regions may be provided in series to improve enrichment and purity or in parallel to improve throughput. It is not uncommon to have five or more parallel multi-stage MFG separation stations on a single chip for applications requiring high throughput and purity.

[0035] The devices described herein enable sophisticated research applications such as high throughput library screening, patient care applications such as tumor cell and pathogen detection, as well as numerous industrial applications that involve separating magnetic materials (e.g., quality control applications). In some embodiments, magnetophoretic sorting stations allow fractionation of magnetic samples based on levels of magnetism in the sample. For example, target cells can be fractionated based the level of expression of a particular protein to which magnetic particles bind. Further, certain integrated devices and systems employ one or more "pre-processing" modules upstream of magnetophoretic sorting stations or "post-processing" modules downstream from the sorting stations. Examples of pre-processing modules include sample loading, filtering and tagging modules. Examples of post-processing modules include lysing, signal amplification, and detection modules.

[0036] While this document often uses the term "microfluidic," it should be understood that the principles and design features described herein can be scaled to larger devices and systems including devices and systems employing channels reaching the millimeter or even centimeter scale channels. Thus, when describing devices and systems as microfluidic, it is intended that the description apply equally to some larger scale devices. It should also be understood that when a channel or station is described herein it is understood that the described channel or station may be a single instance of multiple such channels or stations arranged to operate in parallel on a single substrate or on multiple substrates in an integrated system.

[0037] Note that the term microfluidic "device" is generally understood to mean a single entity in which multiple channels, reservoirs, stations, etc. share a continuous substrate, which may or may not be monolithic. A microfluidics "system" may include one or more microfluidic devices and associated fluidic connections, electrical connections, control/logic features, etc. Aspects of microfluidic devices include the presence of one or more fluid flow paths, e.g., channels, that have microfluidic dimensions, e.g., as provided in greater detail below.

[0038] As an introduction, one example of a microfluidic device of this invention is depicted in FIG. **1**. At various points herein, devices such as the one shown in FIG. **1** will be referred to as a "continuous-flow, magnetic activated cell sorters" (CMACS). As shown in the figure, a pattern of microfluidic channels is employed to separate magnetic particles **103** from non-magnetic particles **105**. The microfluidic channels include sample inlet channels **107***a* and **107***b*, a buffer inlet channel **109**, a sorting region **111**, waste outlet channels **113***a* and **113***b*, and a collection channel **115**. Within sorting region **111** multiple magnetic field gradient generators **117** are provided. These "magnetic field gradient generators" are elements that generate magnetic field gradients in a manner sufficient to alter the influence of an applied magnetic field on magnetically labeled species or intrinsically magnetic species in the sorting region by increasing or decreasing the field strength and/or changing the direction of the field. As explained more fully elsewhere herein, these magnetic field gradient generators serve to shape the distribution of the magnetic field gradient experienced by the particles traveling through the sorting region. In one embodiment, these are nickel strips provided within a flow channel of the sorting region itself. Not shown are one or more magnets that provide an external magnetic field in the sorting region. In one embodiment, a pair of permanent magnets such as NdFeB magnets is placed on the top and bottom of the sorting region. In other embodiments, one or more electromagnets may be employed to allow precise control of the field shape and homogeneity. The MFG strips interact with the field produced by the magnet to precisely shape and direct the magnetic field gradient within sorting region **111**.

[0039] During operation, a buffer solution is introduced through buffer inlet channel **109** and a sample solution is introduced through sample inlet channels **107***a* and **107***b*. The sample solution may include magnetic particles and non-magnetic components from a sample being analyzed (e.g., whole cells, cell components, macromolecules, non-biological particles, etc.). The magnetic particles include a capture moiety that selectively binds with a target component in the sample. Typically, the buffer contains substantially no sample or analyte. However, in some embodiments, the buffer may include reagents for facilitating other operations (non-sorting operations) performed in an integrated microfluidics system (e.g., sample amplification or detection). The buffer and sample solution flow through the sorting region in the laminar regime. Effectively, they flow through the sorting region as uniaxial streams, with little or no mixing. The little mixing that does occur is primarily diffusion driven.

[0040] The magnetic and non-magnetic particles entering sorting region **111** through sample inlet channels **107***a* and **107***b* experience a strong magnetic field gradient imposed by the magnet and MFG strips **117**. The gradient has no effect on non-magnetic materials, so the force on non-magnetic components **105** is primarily in the direction of the F_{drag} arrow in FIG. **1**. This is due to the uniaxial flow of the sample solution along the outer edges of sorting region **111**. Magnetic par-